2010 Invertebrate Monitoring at Duwamish Waterway Restoration Sites: Hamm Creek, Herring's House, Northwind's Weir, and Kenco Marine

Jeffery R. Cordell
Jason Toft

Wetland Ecosystem Team
University of Washington
School of Aquatic and Fishery Sciences

March 2012

Prepared for the United States Fish and Wildlife Service

List of Figures

List of Tables

Summary

This report details the results of invertebrate sampling conducted in 2010 at four restored wetland sites in the industrialized Duwamish River estuary in Seattle, Washington. These sites were constructed with habitat development funds from the settlement of a lawsuit designed to recover damages for injuries to natural resources caused by the release of hazardous substances. With these funds, managed by the US Fish & Wildlife Service, biological monitoring has been occurring at the sites since 2001 shortly after the first site was completed. The goal of the 2010 monitoring project was to quantify insects and benthic invertebrates at the restored sites and at several reference sites in order to provide information about the biological state of the restored sites. Sampling took place once monthly in April, May, and June 2010. We measured insects in emergent vegetation, using fallout traps that catch insects falling from the air or from the vegetation. Benthic organisms (macrofauna and meiofauna) in both mudflat and vegetated habitats were sampled using plastic sediment cores. The main results of the monitoring are as follows:

- At the Herring's House restoration site, which is located in the lower estuary and consists of a constructed embayment off of the main Duwamish Waterway channel, insect taxa richness was significantly higher than at the nearby reference site, and overall insect assemblages were similar to those at the reference site. In contrast, benthic macro- and meiofauna taxa richness values were significantly lower at the restoration site compared to the reference site, and harpacticoid copepod densities were also significantly lower than at the reference site.
- Three restoration sites located in the upper estuary (Hamm Creek, Kenco Marine, Northwind's Weir) were similar to each other in fallout invertebrate taxa richness, and they all had significantly higher taxa richness compared to the reference site. However, the reference site had significantly higher densities of dipteran flies compared to the restored sites in May and June. The insect assemblage at the reference site was different from all of the restored sites, mainly due to large numbers of Acari (mites) and Collembola (springtails) at all three of the restoration sites.
- At the upper estuary restored sites, highest benthic taxa richness values generally occurred at restored sites, especially at the newest site, Kenco Marine which had the highest taxa richness in mudflat habitats on each sampling date. This site also had significantly higher densities of the salmon prey amphipods and harpacticoids and the vegetated stratum at this site often had higher abundances of larval dipterans compared to the other sites. The Hamm Creek restoration vegetated site also had larval dipteran densities that were significantly higher than the reference on two to the three sampling dates.

Most of the results were very similar to those found at the same sites in 2007. This suggests that the invertebrate communities at the restoration sites are stabilizing. For the upper estuary sites, the results of the monitoring also suggest that the restoration sites provide beneficial functions to the estuary in the form of relatively diverse and abundant invertebrates that are potential prey for juvenile salmon. The same is true at the lower estuary Herring's House restoration site for results from the fallout trap invertebrates, but not for benthic invertebrates. The latter finding may be because of physical differences between restoration and reference sites: the Herring's House restored site is relatively enclosed and connected to the Duwamish River through a narrow channel, while the reference site is exposed to the waterway.

Likewise, the differences we observed in both macro- and meiofaunal benthic organisms between the Kenco Marine restoration site and the reference site may be due to their respective physical settings. The restoration site is located further downstream, at the head of the dredged, wider portion of the waterway where there is less river current and more tidal activity, while the reference site is in a more riverine habitat and has experienced recent disturbances in connection with construction of a new restoration site. This underscores the problem of identifying appropriate reference sites in highly urbanized settings where little natural habitat remains, and one alternative is to use older more established sites that have experienced a decade or more of development.

Introduction

The Duwamish Waterway is an industrialized estuarine channel located in Seattle, Washington on the former site of the lower Duwamish River and its associated tidal wetlands. The development of Seattle into a densely populated urban center resulted in the loss of 98% of Duwamish River estuarine wetlands, and replaced them with over 2,100 ha of developed shorelines and floodplain (Simenstad et al., 2005). The Duwamish watershed has also been reduced significantly by permanent diversion of two of three major tributaries, resulting in loss of 70–75% of the historic freshwater inflow to the estuary. The Duwamish estuary has been further degraded by pollution from agriculture, sewage and industrial chemicals, ultimately resulting in the estuary being designated a major Superfund (Comprehensive Environmental Response, Compensation, and Liability Act [CERCLA]) site.

Despite these problems with habitat quantity and quality, restoration of natural functions in the Duwamish estuary is a high priority for trustees of the damaged public resources (Simenstad et al., 2005). Beginning in the early 1990s, mitigation for the loss of the system's wetlands accelerated, and a number of wetland restoration sites have been constructed. These projects have mainly focused on habitats that provide juvenile salmon with food and refuge. Created habitats have consisted mainly of removal of shoreline armoring and other structures in middle and upper intertidal elevations, excavating off–channel features, and planting emergent and riparian vegetation. Between 1992 and 1996, the Federal Coastal America Program funded the first non–regulatory restoration actions in the estuary, coordinated by several federal and local agencies. These sites only comprised 0.5 ha, but they provided the foundation for two clusters of restoration sites that have emerged in the estuary (Simenstad et al., 2005). After 2000, CERCLA actions began appearing, expanding the dimensions and distribution of restoration sites in the estuary. Some of these sites have been periodically monitored for biological attributes important to juvenile salmon and for presence of the salmon themselves, and they appear to be productive relative to reference sites (Cordell et al., 2001, Cordell et al., 2011). The majority of this monitoring has involved indirect measures of productivity, such as amounts of potential prey invertebrates at the sites. This type of "opportunity" measure appraises the capability of juvenile salmon to access and benefit from a habitat, and in this study we adopted those techniques to make post-construction assessments of four recently restored sites in the Duwamish Waterway.

The sites monitored in this study were constructed with habitat development funds from the settlement of a suit against the City of Seattle and Metro (now the King County Department of Metropolitan Services). The purpose of the suit was to recover damages for alleged injuries to natural resources caused by the release of hazardous substances, particularly harmful metals and organic chemicals, from sewer overflows and storm drains. The following site descriptions are in part summarized from the National Oceanic and Atmospheric Administration Damage Assessment and Restoration Program Website at http://www.darrp.noaa.gov/northwest/elliott/restore.html. This website also has a site map showing the location of the restoration sites on the Duwamish River. The sites occur in two areas of the Duwamish River estuary (Fig. 1). The Herring's House restoration and reference sites are in the lower estuary, and the Hamm Creek, Kenco Marine, and Northwind's Weir restoration and reference sites are in the upper part of the estuary.

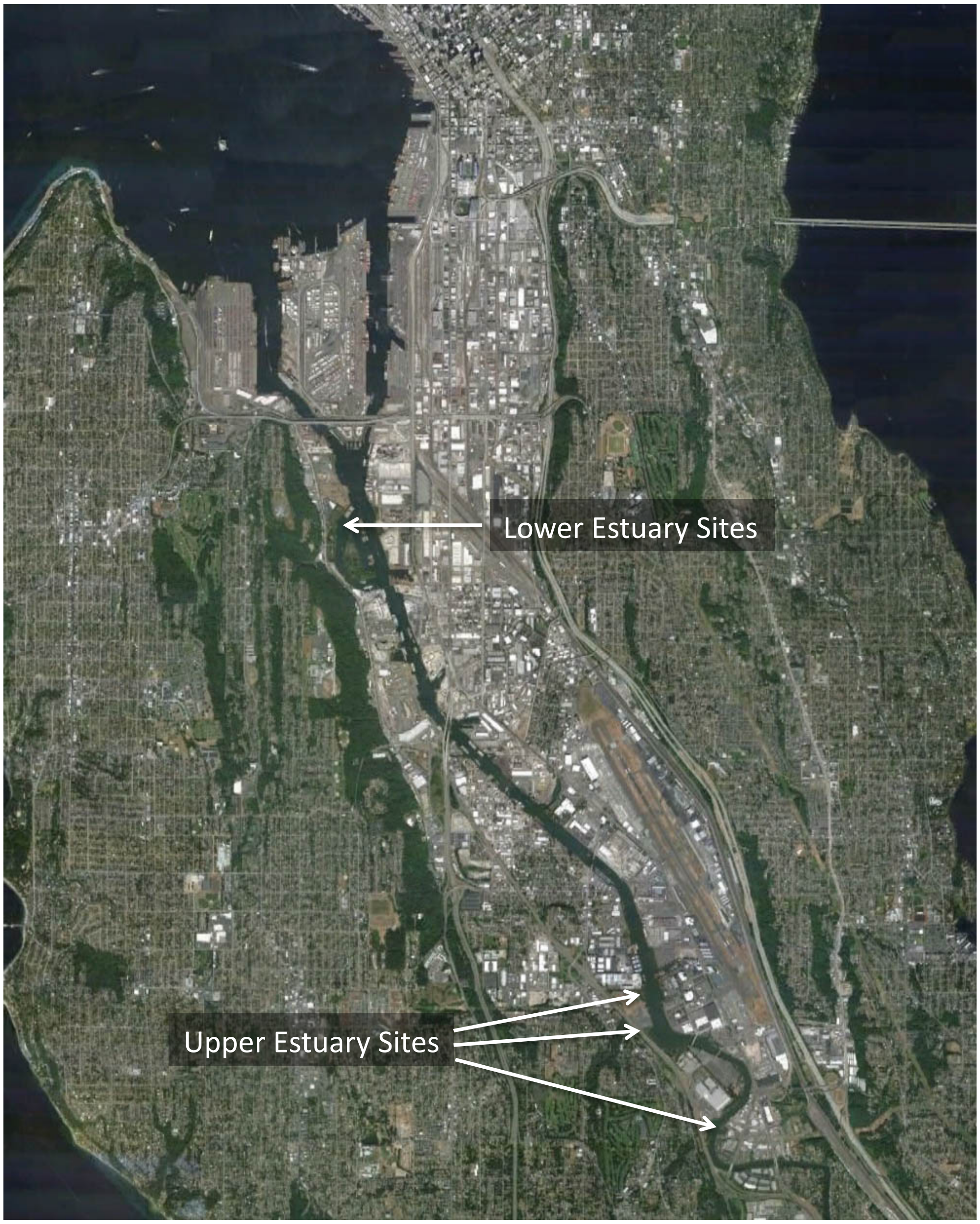

Figure 1. Google Map image showing general areas of restoration sites in the Duwamish River estuary.

The Herring's House restoration project is located at river mile 2 on the site of the former lumber mill that operated from around 1929 until the early 1980's. The site is on the only relict river oxbow, and near Kellogg Island, which is one of the only undeveloped habitat patches in the system. The restoration site is approximately 17 acres in size, with six acres of upland and eleven acres of intertidal habitat. An outer berm consisting of 8-9" quarry stone and fish rock (fine/medium gravel and coarse sand to 3/8 inches) was constructed in 1999. Structures, pilings, paving, and highly contaminated soil were removed, and clean soil and containment features were added. A 1.8-acre intertidal bay of elevations between +6 to +12 feet MLLW was excavated, and protected by two armored spits forming a mouth opening to the Duwamish River.

An amended on-site soil mixture of silts and clays with a high organic content was distributed to a depth of 18 inches over the basin, and the slopes were planted with emergent marsh plants at various elevations. Native scrub/shrub riparian vegetation was planted on the banks and uplands. The primary goal was to provide juvenile salmon with a low-energy intertidal environment that would provide refuge and invertebrate food sources.

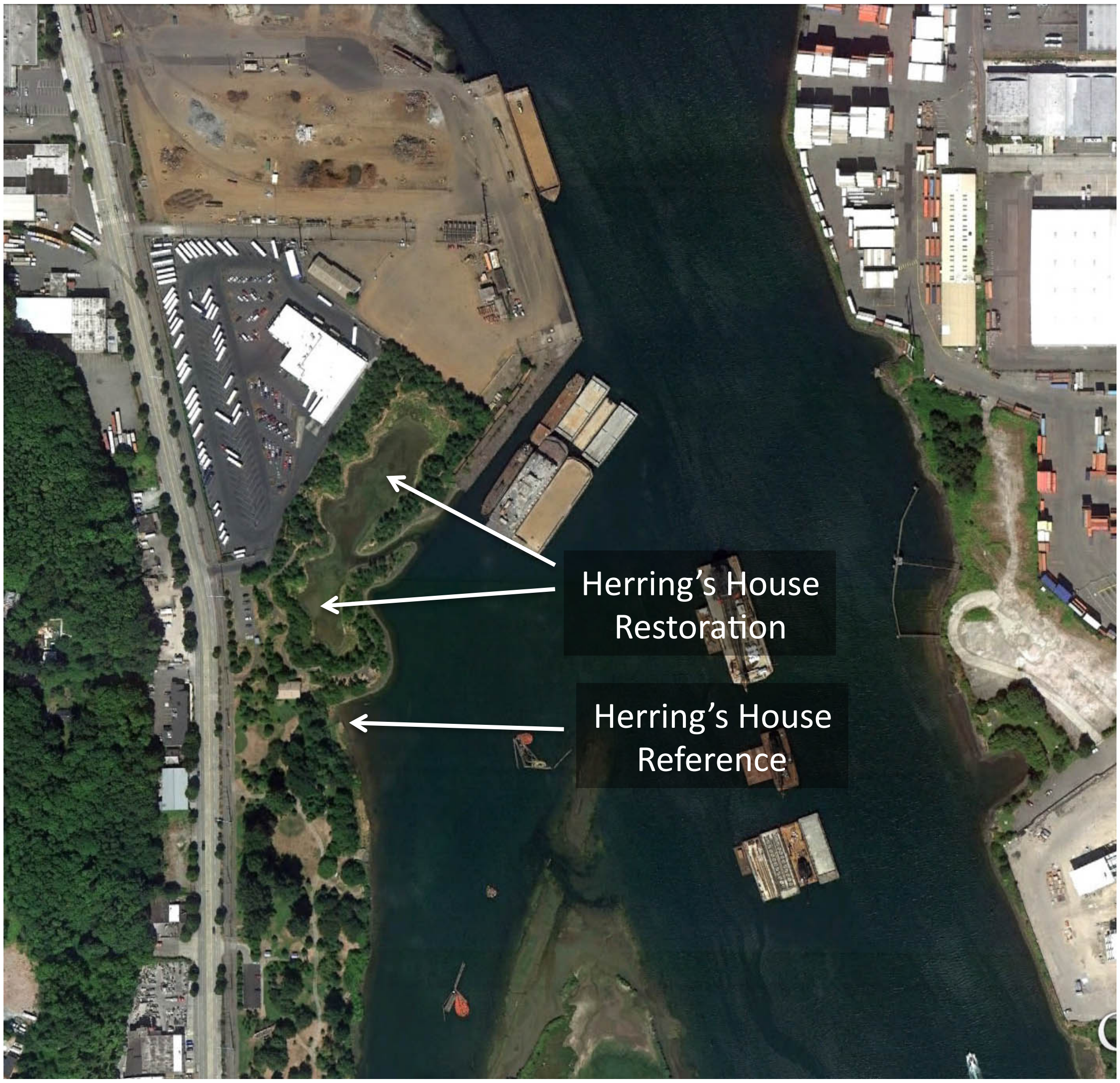

Figure 2. Google Map image showing Herring's House restoration and reference sites.

Hamm Creek is a small stream that joins the west side of the Duwamish River just downstream from the head of the dredged city waterway at approximately river mile 6.2 (Fig. 3). Historically, Hamm Creek meandered through an intertidal marsh before entering the river. From the early 1950's through 1971 the site was used as a dredged material stockpiling area. During this period and prior to the restoration project, it had been routed alongside a road for approximately 1100 feet, and then into a ~1300 foot culvert underneath a boat fabrication business (Delta Marine Industries), and emptied into the river through the same culvert. The restoration effort had the two general goals of 1) restoring important estuarine habitat and 2) improving fish passage and habitat in the freshwater portion of lower Hamm Creek. The restoration site is a 6.2-acre parcel of land within the 21.5-acre Duwamish Substation property owned by Seattle City Light. Construction started in July 1999 and the project was completed in the year 2000. The project was constructed by the U.S. Army Corps of Engineers and King County and consisted of (1) removal of approximately 60,000 cubic yards of historical dredge material at the mouth of the creek, and creating a low-gradient intertidal estuarine wetland and an adjacent freshwater marsh; (2) removing the terminal culvert at the mouth of the creek and "daylighting" the underground portion of the stream; (3) creating a “natural” stream-course, with meanders, fish pools, and large woody debris, for the daylighted section and to replace the straight-line open section of creek that paralleled the road; and (4) planting a new riparian buffer of trees and shrubs along the new stream course. The goals of the project were to provide a more accessible entry to Hamm Creek for salmonid spawning, and to create new riparian stream and intertidal estuarine marsh habitats that will provide refuge and prey resources for juvenile fish.

The North Wind’s Weir project, which was constructed in 2002, is on a 3.1-acre parcel of King County’s Cecil B. Moses Park on the free-flowing Duwamish River about a mile upstream of the end of the navigable channel (Fig. 3). The Elliott Bay/Duwamish Restoration Program purchased 1.03 acres of the park and constructed an intertidal basin by excavating from an elevation of +6 to +15 feet MLLW. The basin is connected to the Duwamish River using natural bank slopes stabilized with vegetation. Upland edges were re-vegetated with native trees and shrubs to form a riparian buffer.

The Kenco Marine site is the newest of the restoration sites, having been completed in April 2006 (Fig. 3). It is located upstream from the Hamm Creek site, and is adjacent to several previously constructed sites at the head of the dredged channel (Turning Basin—see Cordell et al. 2001). The upland portion of the site was composed of fill material covered with asphalt and concrete pads, and several buildings. A commercial pier extended 125 feet into the Turning Basin. Barges and other vessels were moored in the intertidal and subtidal area. Commercial activities and vessels were removed from the site in 1998. In 2005, demolition and removal of buildings, concrete foundations, paved areas, and the dock and associated pilings was completed. Project construction began in September 2005. The area was re-contoured and re-vegetated, creating three habitat benches at various elevations: (1) a lower bench at +2 to +6 feet at a 10:1 slope of sand and gravel created 6,500 square feet of intertidal habitat; (2) an “emergent zone” bench at +9.5 to +11 feet at 20:1 slope was planted with native intertidal vegetation and random rock placement to create 6,050 square feet of marsh habitat; and (3) a riparian zone bench at elevation +14 to +17 feet at a 3:1 slope was planted with native riparian vegetation and created 1,850 square feet of riparian habitat.

Figure 3. Google Map image of Upper Duwamish River estuary restoration and reference sites.

The goal of this monitoring project was to quantify insects and benthic invertebrates at the restored sites and at reference sites within the Duwamish River estuary. Insects produced from riparian and emergent vegetation are often important prey of juvenile salmon foraging in Pacific Northwest estuaries (summarized by Simenstad et al. 1991, Shreffler et al. 1992, Miller and Simenstad 1997, Cordell et al. 2001), including the Duwamish Waterway (Cordell et al. 2001). Likewise, benthic invertebrates that are important prey taxa for juvenile salmon in Pacific Northwest estuaries including the amphipods *Americorophium* spp. and *Eogammarus* spp., larvae of dipteran flies, and harpacticoid copepods (Simenstad et al. 1991, Levings and Nishimura 1997, Cordell et al. 2001, 2006a).

The restoration and reference sites dealt with in this study have been periodically monitored in the past (Table 1). The older Herring's House and Hamm Creek sites were first monitored in 2001, while monitoring at the newer Northwind's Weir and Kenco Marine sites began later. In all cases, monitoring at a given site began one year after construction.

Table 1. Previous monitoring conducted at Duwamish River estuary restoration and reference sites.

Site	Constructed	Years Monitored				
		2001	2002	2003	2007	2010
Herring's House	1999	X	X	X	X	X
Herring's House Reference	NA	X	X	X	X	X
Northwind's Weir	2002			X	X	X
Northwind's Weir Reference	NA			X	X	X
Hamm Creek Estuary	2000	X	X	X	X	X
Hamm Creek Upper Channel	2000	X	X	X	*	*
Hamm Creek Reference	NA	X	X	X	†	†
Kenco Marine	2006				X	X

*Monitoring at Hamm Creek upper channel site ceased after 2003 due to funding constraints.
†Hamm Creek reference site disappeared after 2003 due to erosion and migration of creek mouth: Northwind's Weir reference site was subsequently used as a replacement.

Methods

As in previous sampling efforts at the restoration sites, we characterized invertebrates at the restored and reference sites using fallout traps and benthic macro- and meiofauna core samples (Table 2).

Table 2. Number of invertebrate samples analyzed from three sampling periods at six restoration and reference sites in the Duwamish Waterway in 2010.

Sample Type	Herring's House	Herring's House Reference	Hamm Creek Estuary	Kenco Marine	North Wind's Weir	North Wind's Weir Reference	Total
Fallout Traps	15	15	15	15	15	15	90
Macrofauna Cores							
Vegetated	30	30	30	30	30	30	180
Mudflat	30	30	-	30	30	30	150
Meiofauna Cores	30	30	-	30	30	30	150
Total samples	105	105	45	105	105	105	570

Fallout Traps

We measured insects with a method that has been employed extensively at restoration sites in the Duwamish estuary: simple fallout traps, consisting of plastic storage bins with about 4 cm of soapy water in the bottom (Cordell et al. 2001, 2003, 2006b). They are designed to catch insects that fall from the air or from riparian vegetation and as such measure direct input of insects to the aquatic system. Insects were sampled in April, May, and June, 2010 (weeks of 4/9, 5/18, and 6/15/10). The sampling strata at Hamm Creek consisted of the area of planted emergent vegetation in the restored estuary. At the Herring's House site, the sampling strata were (1) the margins of the created basin that had been planted with emergent vegetation and (2) a natural patch of vegetation (reference site) upstream and adjacent to the site. At the North Wind's Weir site, the sampling strata were (1) the margins of the created basin that had been planted with emergent vegetation and (2) a natural patch of vegetation (reference site) across the river channel from the site. At the Kenco site, the sampling stratum was the "emergent zone" bench at +9.5 to +11 feet that had been planted with emergent vegetation. Five replicate traps were placed haphazardly in or near vegetation at each sampling stratum for a period of three days. At the end of the three-day period, each tray was drained through a 106μm mesh sieve, and the insects were washed into a sample jar and fixed in 70% isopropanol solution. In the laboratory, insects were identified to family level for important salmonid prey taxa, and to order level for the remainder.

Benthic Macro- and Meiofauna

We used a protocol for sampling macro- and meiobenthos in the Duwamish Waterway that has recently been used extensively in the Duwamish Waterway (Cordell et al. 2001, 2003, 2006b). It employs a 2-inch diameter (0.0024 m^2) pvc plastic core taken to a depth of 10 cm for macrofauna and a 1-inch diameter (0.0002 m^2) pvc plastic core taken to a depth of 10 cm for meiofauna. Strata were chosen based on site characteristics. At Hamm Creek, sampling strata consisted of emergent vegetation in the created estuary. Sampling strata at the Herring's House site were (1) the mud/sand flat in the created intertidal basin; (2) the vegetated margins of the basin; (3) a reference flat adjacent to and upriver from the site; and (4) a patch of native vegetation adjacent to the site. Sampling strata at the North Wind's Weir site were (1) the mud/sand flat in the created intertidal basin; (2) the vegetated margins of the basin; (3) a reference flat across the river channel from the site; and (4) a patch of native vegetation across the river channel from the site. Sampling strata at Kenco were (1) the mud and sand lower bench that had been created at +2 to +6 feet and (2) the vegetated margins of the shoreline. For each stratum, 10 replicate cores of each type were taken on 9 April, 18 May, and 15 June 2010. Samples were fixed in the field in 10% buffered formaldehyde solution. Important salmon prey invertebrates were identified to genus or species with the exception of insects, which were identified to family level; other taxa were identified to order level.

Statistical Comparisons Between Restored and Reference Sites

Analyses focused on comparing restoration and reference sites within the lower estuary (Herring's House restored and reference) and the upper estuary (North Wind's Weir reference, and North Wind's Weir, Hamm Creek, and Kenco restored). Analysis of variance (ANOVA; alpha = 0.05) was conducted on average taxa richness and on densities of selected invertebrate categories. For significant results, Tukey's test for multiple comparisons was used to identify specific differences between means.

We used a multivariate analysis to determine assemblage differences in fallout trap and benthic sample data using the PRIMER (Clarke and Gorley 2006) and PERMANOVA (Anderson et al. 2008) statistical programs. A PERMANOVA analysis examines the entire assemblage of species and numbers, and generates a p-value similar to that of a univariate ANOVA test ($p < 0.05$ indicates significant difference). PERMANOVA tests allow for 2-way analysis of site and month, with an interaction term when one factor varies with levels of another other factor. Post-hoc tests in the program identify the specific site pairs that are responsible for significant PERMANOVA results. Assemblage data was log transformed before analysis, and those taxa representing less than 3% of the numbers removed. When significant differences were indicated with PERMAMOVA, a SIMPER (Similarity Percentage) analysis was used to identify the taxa that accounted for the differences. SIMPER is a method for assessing which taxa are primarily responsible for an observed difference between groups of samples, and generates a ranking of the percent contribution by taxa that contribute to the significant differences between factors.

Results

Fallout Invertebrates

<u>Taxa Richness</u>

At the three upper estuary sites, taxa richness was consistently higher at the restored sites as compared to the respective reference sites (Fig. 4). Results of a 2-way ANOVA on site x date on the upstream sites found that both site and sampling month were significant ($p < 0.0001$), and there were no significant interactions. A post-hoc Tukey test indicated that all of the restored sites had significantly higher taxa richness numbers as compared to the reference site. Similarly, at the lower estuary Herring's House sites taxa richness was higher at the restoration site on all three sampling dates, as compared to the reference site. ANOVA indicated that site and sampling month were significant ($p < 0.0001$), and that interactions were also significant ($p < 0.01$). Separate ANOVAs for each sampling month showed that the differences between restored and reference taxa richness were statistically significant in May and June.

Assemblage Compositions

In April, dipteran flies consisting mainly of Chironomidae, Ceratopogonidae, and Dolichopodidae dominated the fallout trap invertebrates at all of the sites (Fig. 4). In May and June the proportion of dipterans was lower, and other taxa such as collembolans and aphids were prominent at several of the sites; collembolans dominated the fallout assemblages at all three upper estuary restored sites in June. The exception to this was the Northwind's Weir reference site, which was dominated by dipterans on all three sampling dates.

A 2-way PERMANOVA conducted on data from the upstream sites showed that Site ($p=0.009$), Month ($p<0.001$) and interaction ($p<0.001$) were all significant. Because of the significant interaction, individual tests were conducted for each sampling month, and results of this indicated that the Northwind's Weir reference site was different from all of the upper estuary restored sites. SIMPER analysis identified higher abundances of collembolans and Acari (mites) at the restored sites as being the main contributors to this difference. Most pairwise tests among the other upper estuary sites also showed that they were significantly different from one another. The main exception to this was at the Hamm Creek and Northwind's Weir restored sites which were not significantly different from each other in May and June.

At the downstream Herring's House sites, PERMANOVA showed that the sites were not significantly different in fallout invertebrate assemblage structure ($p = 0.09$), but there were significant month ($p < 0.001$) and interaction ($p = 0.005$) effects.

Densities

Because diptera as a group were abundant and occurred consistently among the sites, and are prominent juvenile salmon prey, we conducted additional analyses on them. At the upper estuary sites, a 2-way ANOVA on Site x Month showed that site was a significant factor ($p < 0.0001$); month was not significant ($p = 0.21$), and interaction was significant ($p < 0.001$). Separate ANOVAs for each month identified significant differences in dipterans among the sites in May and June ($p < 0.001$). A post-hoc Tukey test indicated that these differences were due to higher diptera densities at the reference site as compared to the restored sites. At Herring's House, there were no significant site, month, or interaction differences in dipterans between the restored and reference sites. However, for densities of all invertebrates combined, a 2-way ANOVA on site x month found that site, month and interactions were all significant. Separate ANOVAs conducted for each month found that the Herring's House restored site had significantly higher total fallout invertebrate densities in May and June.

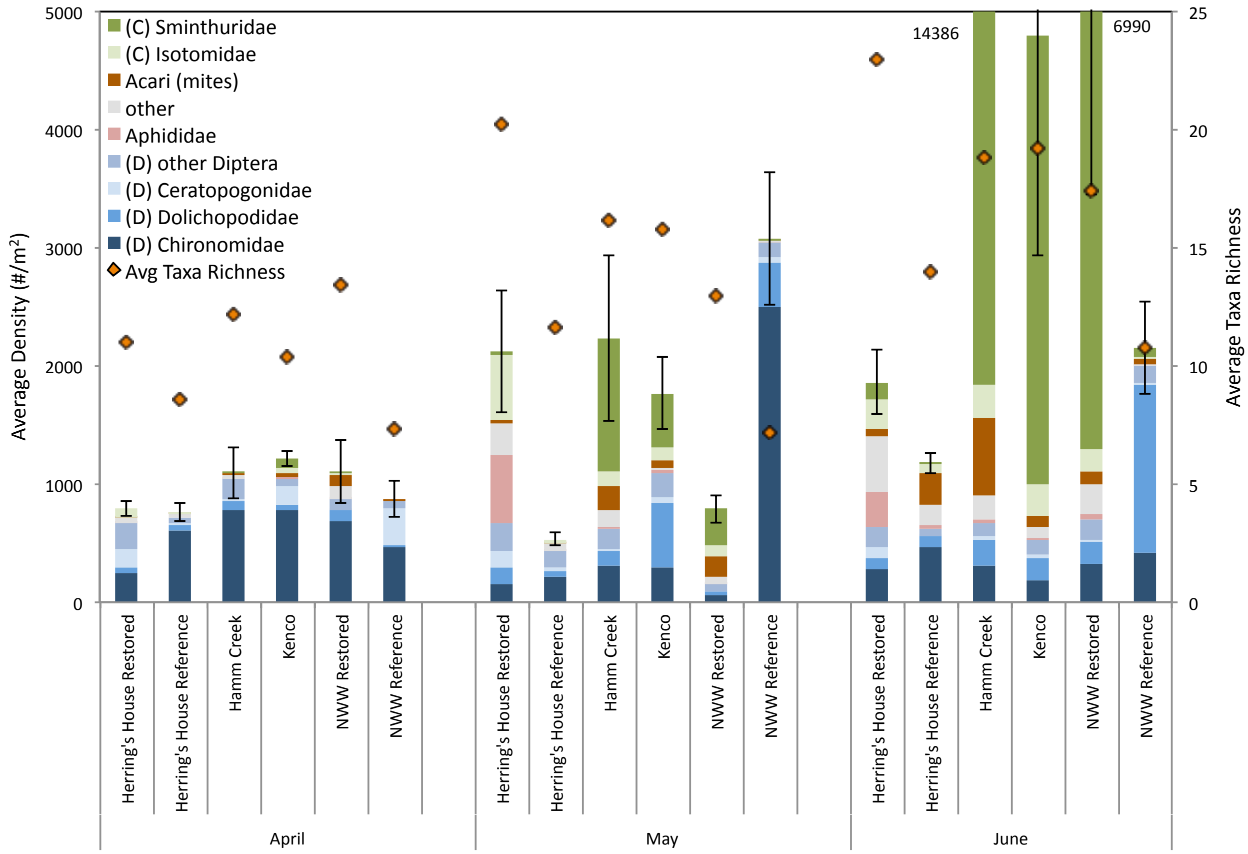

Figure 4. Average density and taxa richness of fallout trap insects and other arthropods. Collembola (springtails) (C) are shaded green; Diptera (midges and other flies) (D) are shaded blue. Error bars represent Standard Error.

Benthic Macrofauna

Upper Estuary Sites

Taxa Richness

On each sampling date, taxa richness was highest at the Kenco Marine mudflat site and lowest at the Northwind's Weir reference mudflat site (Fig. 5). A 2-way ANOVA on site x date indicated that site and sampling month were both significant ($p < 0.0001$); interactions were also significant ($p < 0.001$). Separate ANOVAs conducted by month did not show any consistent significant differences that spanned all sampling periods. However, in April and May the Northwind's Weir reference mudflat site had significantly lower taxa richness than all other sites. Also, the Kenco Marine mudflat site had significantly higher taxa richness than several of the other sites on each sampling date.

Assemblage Compositions

Benthic macrofauna from upper estuary mudflat sites consisted mostly of oligochaetes, nematodes, and polychaetes (Fig. 5). However, the vegetated sites had higher proportions of dipteran larvae than did the mudflat sites. A 2-way PERMANOVA on site x date found that site, month, and interactions were all significant ($p < 0.001$). Subsequent pair-wise tests also found that each site pair was different in each sampling month. SIMPER analysis identified the main taxa contributing to the taxa differences at each site, as follows:

- All mudflat sites were characterized by nematodes and oligochaetes.
- The Northwind's Weir restored mudflat site was also characterized by the polychaete *Hobsonia florida*, chironomid and ceratopogonid fly larvae, and the polychaete *Neanthes limnicola*.
- The Kenco Marine mudflat site was also characterized by the amphipod *Americorophium salmonis,* the polychaetes *Manayunkia aesturina* and *Hobsonia florida*, and the cumacean crustacean *Nippoleucon hinumensis*.
- All of the vegetated sites were characterized by oligochaetes, Acari (mites), and chironomid and ceratopogonid fly larvae.
- The Hamm Creek vegetated site was also characterized by *Manayunkia aesturina*.
- The Kenco Marine vegetated site was also characterized by other types of insect larvae.
- The Northwind's Weir vegetated site was also characterized by *Hobsonia florida*.

Densities of Major Groups

At the upstream sites, the amphipod *Americorophium salmonis* was significantly more abundant at the Kenco Marine mud flat site compared to the other sites (2-way ANOVA, only site significant; post-hoc Tukey test). The Hamm Creek vegetation site had higher polychaete densities (all *Manayunkia aestuarina*) than the Kenco Marine vegetated site, and both Northwind's Weir reference mud and vegetation sites (2-way ANOVA, only site significant; post-hoc Tukey test). For chironomid and other dipteran larvae, a 2-way ANOVA showed that site, month, and interactions were all significant ($p < 0.0001$). Separate ANOVAs conducted for each month, with tukey tests conducted on site when significant differences were found revealed that in April the Kenco Marine vegetated site had higher dipteran densities than the other sites, and the Hamm Creek vegetated site had higher densities than the Kenco marine mudflat and Northwind's Weir reference mudflat sites. In May the Hamm Creek vegetated site had significantly higher densities than the Kenco mudflat site and the Northwinds's Weir reference mudflat and vegetation sites. In June the Kenco Marine vegetated site had higher dipteran densities than the other sites except the Northwinds's Weir restored mudflat site.

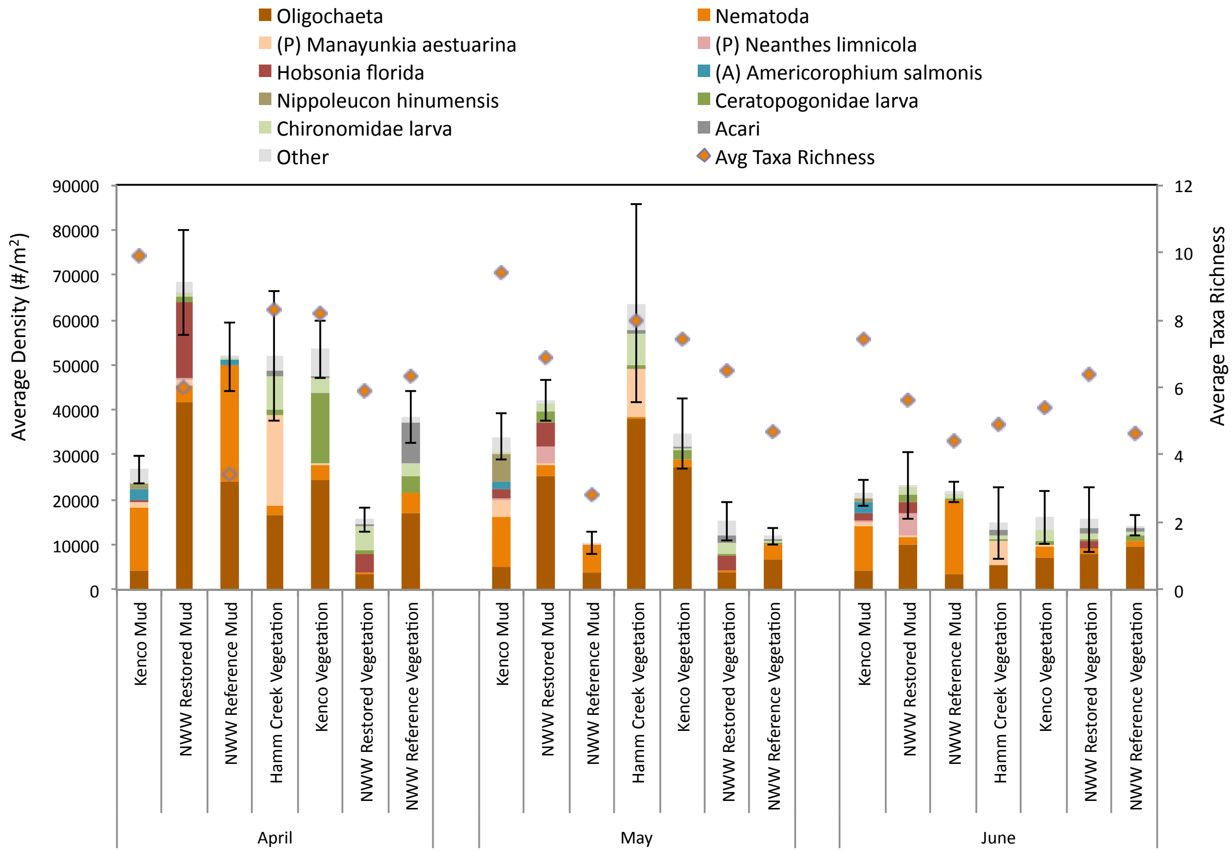

Figure 5. Average density and taxa richness of macroinvertebrates at upper estuary mud flat and vegetation sites. Oligochaete, nematode, and polychaete (P) worms are shaded orange; gammarid amphipods (A) are shaded blue; dipteran fly larva are shaded green. Error bars represent Standard Error.

Lower Estuary Sites

Taxa Richness

On each sampling date, taxa richness was highest at the Herring's House reference mudflat site (Fig. 6). A 2-way ANOVA on site x date indicated that site was significant ($p < 0.0001$), but sampling month and interactions were not. The test found that the higher average taxa richness values at the Herring's House reference mudflat were statistically significant.

Assemblage Compositions

As with the upper estuary sites, benthic macrofauna from the lower estuary mudflat sites consisted mostly of oligochaetes, nematodes, and polychaetes (Fig. 6). However, the lower estuary sites had higher proportions of amphipods. A 2-way PERMANOVA on site x date found that site, month, and interactions were all significant ($p < 0.001$), and pair-wise tests also found that each site pair was different in each sampling month. SIMPER analysis identified the main taxa contributing to the taxa differences at each site. The Herring's House reference mudflat site was characterized by more amphipods as compared to the restored mudflat site, which had more of the polychaete *Hobsonia florida*. At the vegetated sites, the Herring's House reference site was characterized by having more amphipods, while the restored site had more ostracods and ceratopogonid larvae.

Densities of Major Groups

At the downstream Herring's House sites, a 2-way site x month ANOVA on amphipod densities found that site and month were significant ($p < 0.0001$, < 0.01, respectively). Post-hoc Tukey tests showed that the Herring's House reference mudflat site had higher amphipod densities than the other sites. There were no significant differences among the downstream sites in polychaete densities. For dipteran larvae, site, month, and interactions were all significant ($p < 0.0001$, < 0.0001, < 0.01, respectively). Consequently, separate ANOVAs were conducted for each month, with a Tukey test conducted for site when significant results were found. These tests found that the Herring's House restored vegetation site had higher densities than all other sites in April and June, and higher densities than Herring's House reference mudflat site in May.

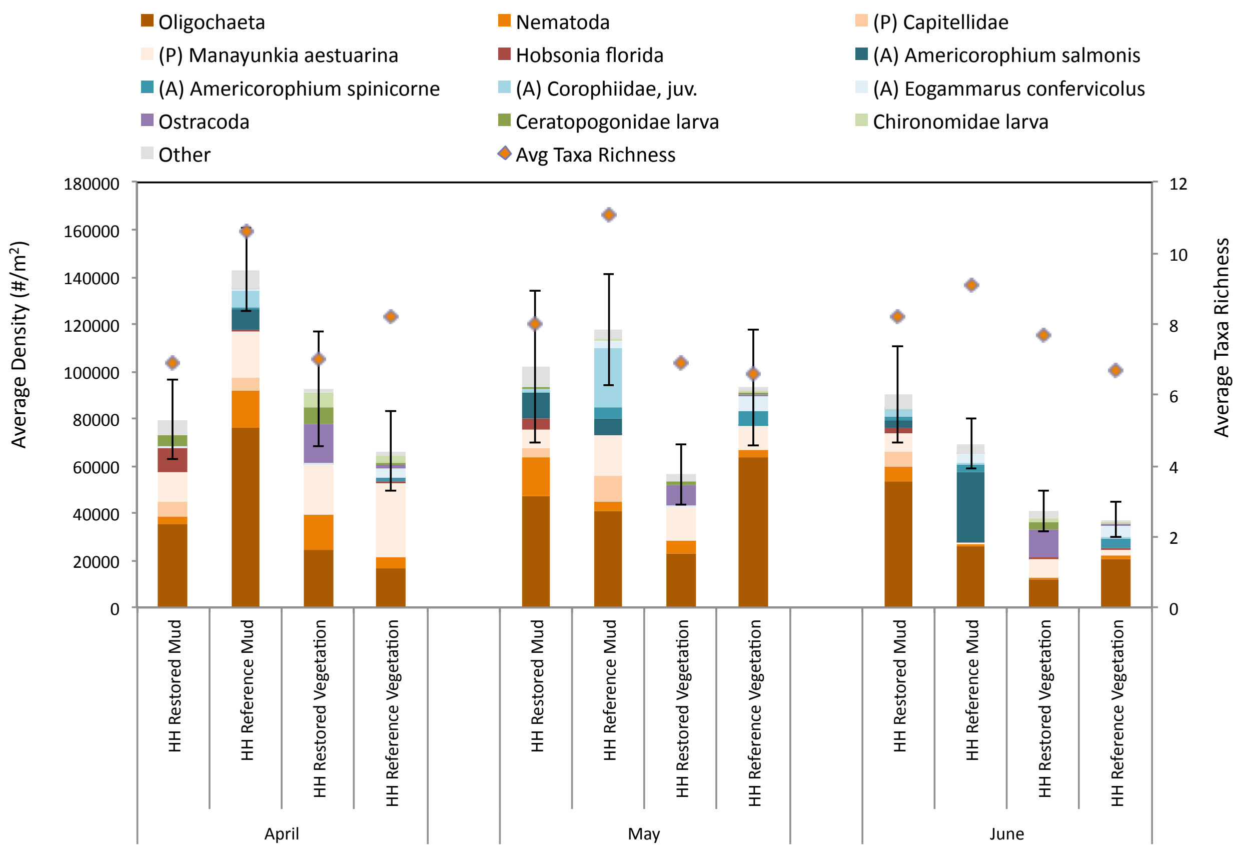

Figure 6. Average density and taxa richness of invertebrates at lower estuary mud and vegetation sites. Oligochaete, nematode, and polychaete (P) worms are shaded orange; gammarid amphipods (A) are shaded blue; dipteran fly larva are shaded green. Error bars represent Standard Error.

Benthic Meiofauna

Taxa Richness

At the lower estuary Herring's House sites average taxa richness was consistently highest at the reference site (Fig. 5). A 2-way ANOVA on site x date found that the only significant factor was site, with average taxa richness being significantly higher at the reference site ($p < 0.0001$).

Among the upper estuary sites, average taxa richness values were more similar than those at the two Herring's House sites, with the highest value occurring at the Kenco Marine site on each sampling date (Fig. 5). A 2-way ANOVA found that site and month were both significant ($p < 0.001$), and interaction was also significant ($p < 0.05$). A post-hoc Tukey test determined that there were no significant differences in April, and that the Northwind's Weir reference site had lower average taxa richness than the Northwind's Weir restored site and the Kenco Marine site in May and June.

Assemblage Compositions

Benthic meiofauna was dominated by oligochaetes, nematodes, and harpacticoid copepods at all of the sites (Fig. 5). Foraminiferans were also prominent at the Herring's House and Northwind's Weir restored sites.

At the upper estuary sites, a 2-way PERMANOVA on site x date indicated that site ($p=0.002$), month and interactions ($p<0.001$) were all significant. Because of the interactions effects, separate tests were done for each month, and these found that in each month, all of the sites were different from each other. A SIMPER analysis was conducted to identify the main sources of difference between the Northwind's Weir reference site and the restored sites. This analysis indicated that the main contributors to the differences were: (1) the Kenco Marine site had more of the harpacticoid copepod *Coullana canadensis* and the polychaete worm *Manayunkia aesturina*; (2) the Northwind's Weir restoration site had more Foraminifera; and (3) the Northwind's weir reference site had more of the harpacticoid copepod *Huntemannia jadensis*.

At the lower estuary Herring's House site, a 2-way PERMANOVA on site x date did not identify any significant differences between the reference and restored sites.

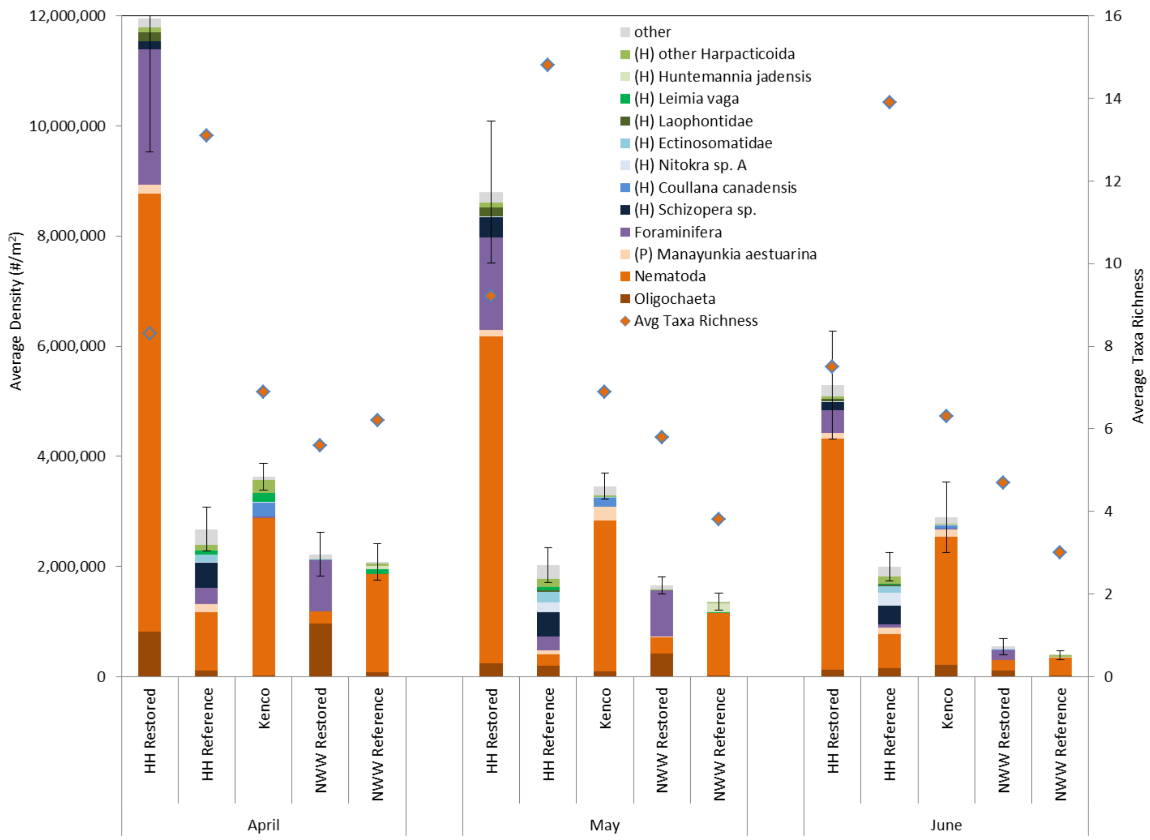

Figure 7. Average density and taxa richness of meiofauna. Oligochaete, nematode, and polychaete (P) worms are shaded orange; harpacticoid copepods (H) are shaded blue and green. Error bars represent Standard Error.

Harpacticoid copepod assemblages differed between the upper and lower estuary sites (Fig. 6). At the lower estuary Herring's House sites *Schizopera* sp., the family Laophontidae, *Nitokra* spp., and the family Ectinosomatidae dominated, while at the upper estuary sites *Coullana canadensis, Leimia vaga*, and *Pseudobradya* sp. were most abundant. For total harpacticoid densities, a 2-way ANOVA on site x month found that at the upper estuary sites site, month, and interactions were all significant ($p < 0.0001$). Post-hoc Tukey tests on each sampling month separately found that the Kenco Marine site had significantly higher harpacticoid densities as compared the Northwind's weir restored and reference sites in April; the Kenco Marine and Northwind's Weir reference sites had higher densities than the Northwind's Weir restoration site in May, and the Kenco Marine site had higher densities than the Northwind's Weir restoration site in June. At the lower estuary Herring's House sites, a 2-way ANOVA found the site was significant ($p < 0.01$), with the reference site having significantly higher harpacticoid copepod densities, while sampling date and interactions were not.

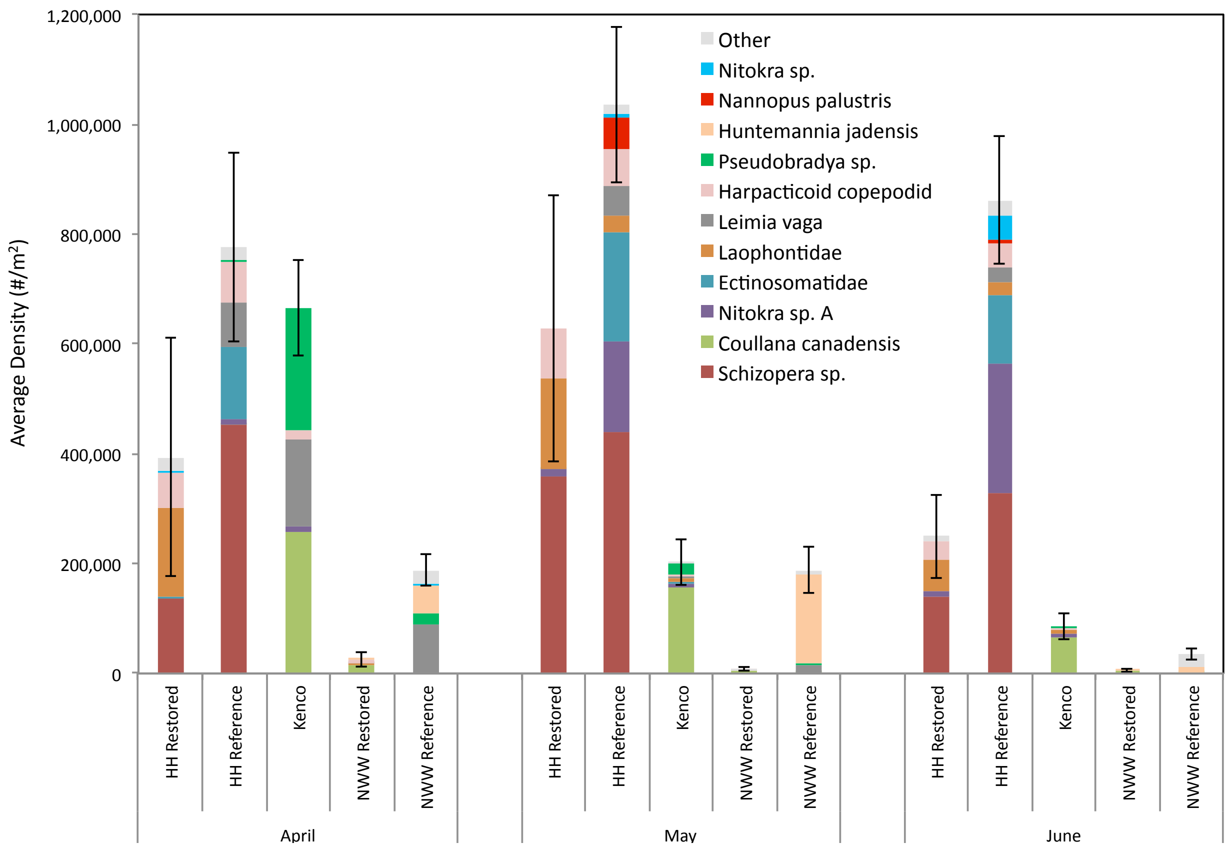

Figure 8. Average densities of harpacticoid copepods. Error bars represent Standard Error.

Discussion

Fallout Insects

Similar to findings from the 2007 sampling, the Herring's House restoration site had higher taxa richness and total invertebrate densities than the reference site (in 2010 significantly higher in May and June). However, in contrast to the 2007 data, the PERMANOVA tests did not find that the fallout trap assemblages were different between the restored and reference sites. While the restoration site did appear to be different from the reference site in having much higher proportions and numbers of aphids, high variability among replicates in this group kept the differences from being statistically significant. These results taken as a whole suggest that the Herring's House restored site has developed insect assemblages that are diverse and abundant compared to reference levels.

The three upper estuary restoration sites were similar to each other in fallout invertebrate taxa richness, and they were significantly higher than those at the reference site. However, the reference site had significantly higher densities of dipteran flies as compared to the restored sites in May and June. This is different from the 2007 monitoring, which found that the Hamm Creek and Kenco Marine restoration sites had higher dipteran densities than the reference site. Another difference from previous monitoring is that the PERMANOVA tests indicated that the Northwind's Weir reference site was different from all of the other sites, mainly due to large numbers of Acari (mites) and Collembola (springtails) at all three of the restoration sites. In

2007, these two taxa were not abundant at the Hamm Creek and Kenco Marine restoration sites. The differences between the reference and restored sites in the 2010 data make it difficult to evaluate the functional state of the restored sites. On one hand salmonid prey dipterans are lower at the restored sites, while on the other, Collembola (which are also salmon prey) are higher at the restored sites. However, in general the restored sites appear to have developed diverse and relatively abundant insect assemblages that could provide juvenile salmon with prey.

Benthic Invertebrates

Similar to the findings from the 2007 monitoring, the Herring's House reference mud flat benthic assemblage had higher taxa richness and more *Americorophium* spp. amphipods, compared to the restoration mudflat site, which had more of the polychaete *Hobsonia florida*. Similarly, the Herring's House vegetated reference site was also characterized by having more amphipods compared to the restoration site. As was discussed in the 2007 report (Cordell et al. 2008), these results are consistent with monitoring findings from restored and reference sites at the Duwamish River Turning Basin, in which *Americorophium* spp. were more abundant in benthic samples and salmon diets from reference habitats, while polychaetes were more prevalent in restored habitats (Cordell et al. 2009, 2011). Similar to the results from the macrofauna samples, the Herring's House restored site had significantly lower taxa richness and harpacticoid copepod densities than the reference site. These are the same results that were found in the 2007 monitoring.

The Herring's House restored and reference site differ in their physical settings, with the restored relatively enclosed and connected to the Duwamish River through a narrow channel and the reference site being exposed to the waterway. As such, there are a variety of factors that could contribute to these differences in biota, including differences in sediment grain size, elevation gradient, flushing rate, and amount of organic material.

At the upper estuary restored sites, highest taxa richness values generally occurred at restored sites as was seen in 2007. This was especially true at the Kenco Marine mudflat sites, which had the highest taxa richness on each sampling date. Also similar to the 2007 results, the Kenco Marine restoration site had significantly higher densities of the salmon prey amphipods *Americorophium* spp. In statistical comparisons, the Kenco Marine vegetated site often differed from the other sites in having higher abundances of larval dipterans, which are another source of prey for juvenile salmon. The Hamm Creek restoration vegetated site also had larval dipteran densities that were significantly higher than the reference on two of the three sampling dates. These findings, along with more general results showing similar or higher densities of organisms at the upper estuary restored sites as compared to the references suggest that the restoration sites are providing beneficial functions to the estuary.

As in 2007, the Kenco Marine restoration site usually had high taxa richness and harpacticoid densities as compared to the North Wind's Weir reference and restoration sites. Also as in 2007, the Kenco Marine restoration site was dominated by three species of harpacticoids (*Pseudobradya* sp., *Leimia vaga*, and *Coullana canadensis*) that were found to be among the most abundant prey harpacticoids in juvenile chum salmon caught at the Duwamish Waterway turning basin near the Kenco Marine site (Cordell et al. 2009). In the 2007 monitoring report we conjectured about whether or not the high harpacticoid abundances would persist at the then new Kenco Marine site, and this does appear to have happened.

The differences we observed in both macro- and meiofaunal benthic organisms between the Kenco Marine site and the Northwind's weir reference site may be due to their respective physical settings, which are quite different. The Kenco Marine site is located downstream of the Northwind's Weir sites, at the head of the dredged, wider portion of the waterway (Fig. 3), and is probably subject to less river current, more tidal activity, and higher deposition of sediments and organic material. Also, the Northwind's Weir restoration site is different than other sites in being at a relatively high elevation and having only a small amount of tidal exchange through a small, narrow channel (Fig 7). In addition to experiencing higher river current velocities, the Northwind's Weir reference site has experienced recent disturbances in connection with construction of a new restoration site (Site 1; Fig. 7). This underscores the problem of identifying appropriate reference sites in highly urbanized settings where little natural habitat remains. In this and previous studies of Duwamish River estuary restoration sites, reference sites have consisted of small, relict patches of native emergent vegetation or mudflats. While they have served as comparisons by which to measure habitat improvements provided by restored sites, it may be useful in future monitoring efforts to consider other types of references. One alternative is to use older more established sites that have experienced a decade or more of development. An example of this in the Duwamish River estuary is at the head of the dredged navigation channel (Turning Basin), where there are several older restoration sites (these can be seen in Fig. 3, just upstream of the Kenco Marine site).

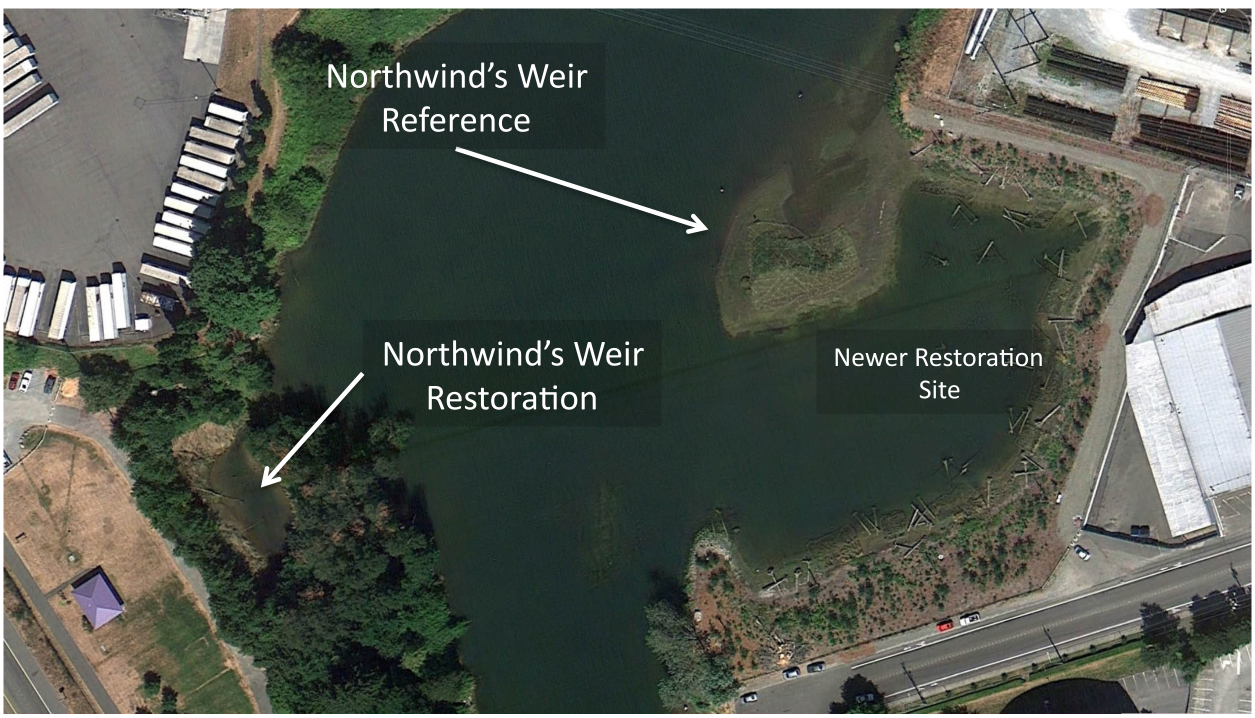

Figure 9. Google Map image showing details of Northwind's Weir restoration and reference sites. Faint lattice patterns at the reference site are goose exclosures.

Literature Cited

Anderson, M.J., R.N. Gorley, and K.R. Clarke. 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK.

Clarke, K. R., and R. N. Gorley. 2006. Primer v6: User Manual/Tutorial. Plymouth, UK: PRIMER-E.

Cordell, J. R., L. M. Tear, and K. Jensen. 2001. Biological monitoring at Duwamish River Coastal America restoration and reference sites: a seven-year retrospective. SAFS-UW-0108, School of Aquatic and Fishery Sciences, Univ. Wash., Seattle, WA. Cordell, J.R., A. Gray, and L.M. Tear. 2003. 2001-2003 invertebrate monitoring at Duwamish Waterway restoration sites: Hamm Creek, Herring's House, and Northwind's Weir. School of Aquatic and Fishery Sciences, Univ. Wash., Seattle, WA.

Cordell, J.R., A. Gray, and L.M. Tear. 2003. 2001-2003 invertebrate monitoring at Duwamish Waterway restoration sites: Hamm Creek, Herring's House, and Northwind's Weir. School of Aquatic and Fishery Sciences, Univ. Wash., Seattle, WA.

Cordell, J., J. Toft, M. Cooksey, and A. Gray. 2006a. 2005 Juvenile Chinook Duwamish River Studies, Study 2: fish assemblages and patterns of Chinook salmon abundance, diet, and growth at restored sites in the Duwamish River. Technical report prepared for WRIA9. 53 pp.

Cordell, J.R., J. Toft, S. Heerhartz, and E. Armbrust. 2006b. 2005 invertebrate monitoring at Duwamish Waterway restoration sites: Hamm Creek, Herring's House, and Northwind's Weir. Technical report prepared for United States Fish and Wildlife Service. 52 pp.

Cordell, J.R., J. Toft, and E. Armbrust. 2009. Fish and invertebrates at a wetland restoration site in the Duwamish River estuary, Seattle, Washington: results of biological monitoring at Turning Basin number three, 1999-2007. Draft technical report prepared for the Port of Seattle. 53 pp.

Cordell, J. R., Toft, J. T., Gray, A, Ruggerone, G. T., and Cooksey, M. 2011. Functions of restored wetlands for juvenile salmon in an industrialized estuary. Ecol. Engr. 37:343-353.

Levings, C.D. and D.J.H. Nishimura. 1997. Created and restored marshes in the lower Fraser River, British Columbia: summary of their functioning as fish habitat. Water Qual. Res. J. Can 32:599:618.

Miller, J.A., and C.A. Simenstad. 1997. A comparative assessment of a natural and created estuarine slough as rearing habitat for juvenile Chinook and coho salmon. Estuaries 20(4):792-806.

Shreffler, D.K., C.A. Simenstad, and R.M. Thom. 1992. Juvenile salmon foraging in a restored estuarine wetland. Estuaries 15:204-213.

Simenstad, C.A., C.D. Tanner, R.M. Thom, and L.L. Conquest. 1991. Estuarine Habitat Assessment Protocol. Report to U. S. Environmental Protection Agency, Region 10, Seattle, Washington. EPA 910/9-91-037. 201 p + appendices.

Simenstad, C.A. and J.R. Cordell. 2000. Ecological assessment criteria for restoring anadromous salmonid habitat in Pacific Northwest estuaries. Ecol. Eng. 15:283-302.

Simenstad, C. A., C. Tanner, J. Cordell, C. Crandell and J. White. 2005. Challenges of habitat restoration in a heavily urbanized estuary: Evaluating the investment. J. Coast. Res. 40: 6-23.